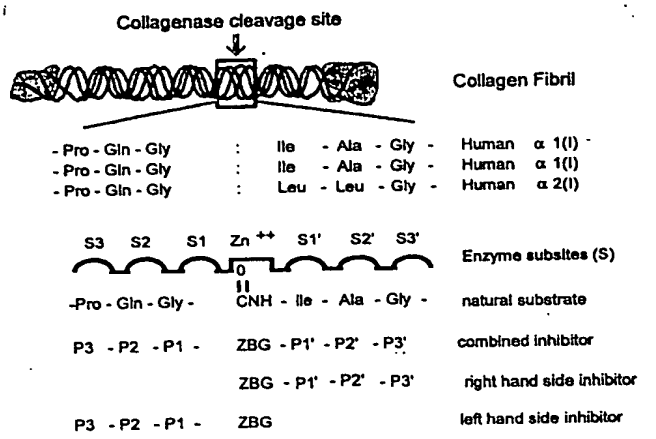

Figure 5. Design of matrix metalloproteinase inhibitors based on the sequence of the collagen substrate cleavage site.

attention[121–123] as right-hand side MMP inhibitors. The early investigations resulted in the identification of left-hand side hydroxamates with only micromolar inhibitory activity such as Z–Pro–Leu–Ala–NHOH (**2**).[119,120]

A key question that medicinal chemists working in the area have tried to answer is "which ZBG is best"? This issue has been addressed by comparing different ZBGs while keeping the rest of the inhibitor structure constant. Using this approach, Castelhano and co-workers arrived at the following preference in terms of inhibition of MMP-1: hydroxamate (e.g., **3**) ≫ formylhydroxylamine > sulfhydryl > phosphinate > aminocarboxylate > carboxylate.[124] Comparison of X-ray crystal structures of **3** and its corresponding carboxylate and sulfodiimine analogues bound to MMP-7 emphasizes the dominant role the ZBG plays in determining the inhibitory potency. The geometries of various ZBGs have been reviewed previously.[76] The hydroxamate acts as a bidentate ligand with each oxygen an optimal distance (1.9–2.3 Å) from the active-site zinc(II) ion, and the position of the hydroxamate nitrogen suggests that it is protonated and forms a hydrogen bond with a carbonyl oxygen of the enzyme backbone. As will become apparent from the discussion below, optimization of the inhibitor structure for many of the above ZBGs can lead to nanomolar inhibition of selected MMPs.

We consider that there are four broad classes of MMP inhibitors as follows: (a) Succinyl hydroxamates, (b) Sulfonamide hydroxamates and related structures, (c) Non-hydroxamates, and (d) Natural products and their derivatives.

While this classification is somewhat arbitrary, it does, we feel, reflect the structural classes of MMP inhibitors that have been investigated. Each of these classes are discussed in turn below with particular emphasis on more recent results.

A. Succinyl Hydroxamates

Early studies conducted by Johnson and co-workers demonstrated that succinyl hydroxamic acid derivatives (e.g., **4**) are more potent inhibitors of MMP-1 than either the corresponding malonyl (e.g., **5**) or glutaryl derivatives (e.g., **6**).[114] The insertion of a single methylene spacer between the ZBG and the carbon bearing the P1′ substituent also showed an improvement in activity for other ZBGs (thiol, formylhydroxylamine, and phosphonate) investigated.[114]

Interestingly, while X-ray analyses of enzyme/succinyl hydroxamate inhibitor complexes have revealed substrate-like binding modes, X-ray analysis of a malonyl hydroxamate bound to MMP-8 reveals a nonsubstrate-like binding mode.[83] It was discovered that the peptidic tail of the weak inhibitor (2S)-HONH–Mal(i-Bu)–Ala–Gly–NH_2 (**7**) bound into the deep S1′ pocket of MMP-8.[83] This insight has led to the discovery of more potent nonpeptidic malonyl hydroxamates (e.g., **8**),[125–127] but the inhibitory activity of these compounds is not as great as that generally obtained with succinyl hydroxamates. The succinyl hydroxamates may be subdivided into peptidic derivatives that possess a P2′ amino acid residue and non-peptidyl compounds in which this group is not an amino acid.

1. Succinyl Hydroxamates with a P2′ Amino Acid Residue

Independently of Johnson and co-workers,[114] the group of Dickens also disclosed that succinyl hydroxamates, such as SC-44463 (**9**), possess potent MMP inhibitory activity.[128] The structure–activity relationships (SAR) for succinyl hydroxamates possessing a P2′ amino acid residue have been extensively explored. An overview of the SAR is given in Figure 6 and discussed in more detail below.

a. P1 Modifications. We and others have found that generally the introduction of a P1 substituent (substituent α to hydroxamic acid) confers the property of broad-spectrum activity against a variety of MMP enzymes.[129] A beneficial effect is conferred by both lipophilic substituents and those capable of undergoing hydrogen bonding. A P1 alkylcyclic imido substituent increases the potency of not only succinyl hydroxamate MMP inhibitors but also MMP inhibitors with amino carboxylate, phosphinate, and phosphonic acid ZBGs. The role of the cyclic imido group is not entirely clear. In MMP-1, analysis of an X-ray crystal structure has shown that the S1 asparagine (Asn-180; MMP-1 numbering) residue hydrogen bonds to the carbamate carbonyl group of an amino carboxylate inhibitor that possesses a P1 benzyloxycarbonylaminoalkyl substituent.[77] A similar interaction is observed between the carbonyl of a P1 phthalimido methyl substituent and Asn-180 in the X-ray structure of Ro 32–0554 (**10**) complexed to MMP-1 catalytic domain.[130] However, a beneficial effect of a P1′ substituent is often observed for the inhibition of MMP-3 even though the corresponding residue to the asparagine is valine which cannot partake in such a hydrogen bonding interaction.

In our own research program, investigation of P1 substituents led to the discovery of batimastat (BB-94) (**1**),[131,132] BB-1101 (**11**),[133] and later marimastat (BB-2516) (**12**).[129,134] Batimastat possesses a thienylthiomethylene α-substituent and BB-1101 features a smaller allyl α-substituent, while the α-substituent for marimastat is a hydroxyl group. All three compounds are broad-spectrum inhibitors which have

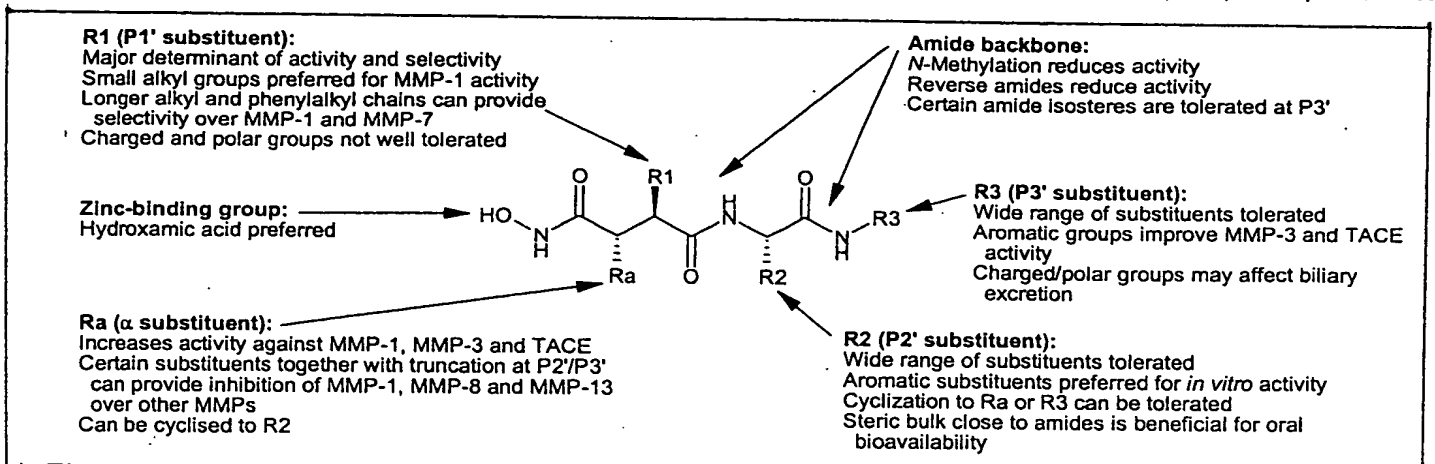

Figure 6. Summary of structure–activity relationships for right-hand side MMP inhibitors.

displayed efficacy in animal models of human disease (vide infra). Unlike marimastat, batimastat and BB-1101 are not orally available. We have attributed the oral availability of marimastat in part to the increase in aqueous solubility achieved by the introduction of the α-hydroxyl group. An X-ray crystal structure of BB-1909 (**13**), an analogue of marimastat, complexed to the catalytic domain of human neutrophil collagenase reveals that the hydroxyl is directed away from the protein surface and is hydrogen-bonded to a solvent molecule.[135] We later found that the presence of certain α-substituents such as allyl (as in BB-1101) and thienylsulfonylmethylene (as in BB-3103 (**14**)) had a beneficial effect on the inhibition of TACE. This dual activity may be of benefit in diseases which involve both inflammation and matrix remodeling and has been implicated in the pharmacological activity of succinyl hydroxamate compounds, such as BB-1101 (**11**) in animal models of arthritis[136] and multiple sclerosis.[137]

Recently, analogues of marimastat have been reported in which the α-position is disubstituted, e.g., **15**.[138] These compounds feature an α-hydroxy group and an α-methyl group and there is a strong stereochemical preference at the α-position, which is opposite to that of marimastat with respect to the orientation of the hydroxyl.[138] Furthermore, phenylpropyl is reported to be the optimal substituent at P1′ and, rather surprisingly, provides potent inhibition of both the short pocket enzyme MMP-1 and the deep pocket enzymes MMP-3 and MMP-9. An X-ray crystal structure of **15** complexed to the catalytic domain of MMP-3 reveals a hydrogen bond between the α-hydroxy group and the backbone of Ala-165 (MMP-3 numbering), as predicted by modeling, and also a van der Waals (VDW) interaction between the P1′ aryl group and His-201. The preparation of the succinate portion of these compounds has been published by the Evans group and involves the catalytic asymmetric aldol reaction between methyl pyruvate and the appropriate enolsilane.[139] Gem-disubstitution at the α-position has also been reported for analogues of BB-1101 (**11**).[140] The combination of α-methyl and α-allyl with *S* stereochemistry (e.g., **16**) is well tolerated, whereas activity is reduced when the stereochemistry at the α-position is *R* and by *gem*-diallyl substition.[140] The pharmacokinetics were investigated for compound **16**, but it was found that the quarternary α-carbon did not confer any benefit compared to BB-1101. We have replaced the α-hydroxy group of marimastat, respectively, with an α-alkoxy, e.g., **17**,[141] and an α-cycloalkyl group, e.g., **18**.[142] Compound **18** is orally available in the rat and the marmoset and inhibits TNF-α production following oral administration in a rat lipopolysaccharide (LPS) challenge model.[142] The 2,3-disubstituted succinate of compound **18** was prepared by a stereoselective Ireland–Claisen rearrangement.[142,143] The synthesis by solid-phase methods of marimastat analogues in which the α-hydroxy group is replaced by a substituted α-amino group has been recently reported.[144]

From analysis of the X-ray crystal structure of batimastat complexed to the active site of recombinant MMP-8 catalytic domain, it is apparent that the α-thienylthiomethylene substituent points away from the enzyme as does the P2′ phenylalanine side chain.[84] Similarly, it has been observed in the X-ray structure of BB-16 (**19**) complexed to MMP-3 catalytic domain that the P1 and P2′ substituents are directed away from the active site into solvent.[145] These observations suggest the possibility of joining the P1 and P2′ side chains together to form a cyclic inhibitor. Similar cyclization strategies have been followed by Xue and co-workers[145] and by Steinman and co-workers.[146] Both groups identified the same compound, SE205 (**20**), as possessing similar potency to uncyclized analogues. Interestingly, this cyclization strategy resulted in a substantial increase in aqueous solubility (SE205 13 mg/mL vs BB-16 0.3 mg.ml).[145] Increasing the ring size by insertion of one or two methylenes in the alkyl chain from the α-position was well tolerated.[146] Similar inhibitory activity was obtained for the 13-member amide-linked derivative SC903 (**21**).[145] Alternative P1 to P2′ macrocyclization strategies have been reported that involve amine formation.[147,148] Depending on the nature of the macrocyclic amine, a degree of selectivity can be obtained for MMP-9 and MMP-8 over MMP-1 and MMP-3[147] or activity enhanced against TACE.[148] The introduction of conformational restraint by the construction of a three-membered ring between the α and P1′ positions (e.g., **22**) has been reported by Martin and co-workers to result in a reduction in the

inhibition of MMP-9.[149] Ghose and co-workers investigated a variety of approaches for the introduction of conformational restraint into the succinyl group in order to determine the pharmacophoric geometry for MMP-1 inhibition.[150] A cyclopropyl derivative (**23**) that possesses improved in vitro potency in comparison to **22** was identified in this study.[150] The introduction of a six-membered ring between the α and P1′ positions (e.g., **24**) resulted in ineffective compounds.[151]

b. P1′ Modifications. As discussed earlier, the S1′ pocket is considered to be the selectivity pocket for the MMP inhibitors. This is confirmed by SAR data which shows that certain MMPs tolerate large hydrophobic P1′ side chains: a P1′ 3-phenylpropyl group provides selective inhibition of MMP-2 over MMP-1 and MMP-3 for succinyl hydroxamates (e.g., **25**)[152] and carboxylates and for phosphonate ZBG MMP inhibitors.[153] This seminal discovery by Porter, Morphy, and co-workers was made before structural data on the MMPs revealed that the S1′ subsite is a deep pocket for the majority of the enzymes (e.g., MMP-2, MMP-3, MMP-8, etc.) but is occluded for a few of the MMPs (e.g., MMP-1 and MMP-7) (vide supra). It is intriguing that compound **25**, one of the first deep pocket selective MMP inhibitors, should show greater potency for the inhibition of MMP-2 over MMP-3. This tendency for lower IC_{50} values against MMP-2 (and the other gelatinase MMP-9) than MMP-3 is exhibited by the majority of MMP inhibitors with extended P1′ groups. The discovery that the incorporation of extended P1′ groups can provide potent deep pocket selective MMP inhibitors has been embraced and enhanced by medicinal chemists working in this field. An extended alkyl group at P1′ provides deep pocket selectivity. Broadhurst and co-workers showed that a C_9 alkyl chain at P1′, as in compound **26**, gives reduced in vitro inhibition of MMP-1 while maintaining potent inhibitory activity against MMP-2, MMP-3, and MMP-9.[154] For a series of matlystatin derivatives, a C_9 alkyl chain at P1′, as in R-94138 (**27**), provides at least 10-fold greater potency than C_8 or C_{10} for the inhibition of MMP-9.[155] In analogous succinyl hydroxamates featuring a *n*-nonyl P1′ substituent, the C_9 chain length provides at least a 500-fold selectivity for MMP-2 inhibition over inhibition of MMP-1[107,154] yet extending the P1′ substituent to C_{10}, as in compound **28**, results in potent inhibition of MMP-1.[107] Increasing the length of the P1′ side chain further to C_{16}, as in compound **29**, results in a loss of activity against MMP-1.[107] A similar switch in the inhibition of MMP-1 has been observed within a series of succinyl hydroxamate MMP inhibitors with extended P1′ substituents containing heteroatom-based modifications.[156−158] The benzyl ether **30** is a weak MMP-1 inhibitor, whereas the corresponding phenyl ether **31** is a potent MMP-1 inhibitor.[156−158] These results indicate that selected extended P1′ substituents can be accommodated in the S1′ pocket of MMP-1. In the case of compound **31** it has been proposed that the S1′ blocking residue of MMP-1, Arg-214 (MMP-1 numbering), might be displaced by a $\pi-\pi$ interaction between the electron-rich phenolic group and the electron-poor guanidinium group.[158] Thus, the occlusion of the S1′ pocket for MMP-1 (and presumably that for MMP-7 and MMP-11) is not absolute since the pocket can undergo conformational changes to accommodate certain extended P1′ substituents.[76,91] Interestingly, disubstitution at the α-position has been observed to increase MMP-1 potency for a P1′ 3-phenylpropyl compound (**15**)[138] in comparison to a des-α analogue (e.g., **25**).[152] Biphenylalkyl P1′ substituents have been incorporated into MMP inhibitors with amino carboxyl and carboxylic acid ZBGs.[76,159,160] This modification has also been successfully applied to succinyl hydroxamate compounds (e.g., **32**). Similar deep pocket selectivity is observed for MMP inhibitors that feature the related rigid arylalkynylmethylene P1′ substituents as in compound **33**.[161]

In a series of α-unsubstituted succinyl hydroxamic acid derivatives, phenyl, benzyl, or 2-naphthylmethyl are P1′ substituents of choice for the inhibition of soluble CD23 formation.[162] Selectivity for the inhibition of soluble CD23 formation over inhibition of MMP-1 has been achieved by the combination of P1′ benzyl with an oxime group at P1 as in compound **34**.[163] P1′ phenyl substitution has also been reported for succinyl hydroxamic acid MMP inhibitors by Robinson and co-workers.[164] They also investigated P1′ C-α gem-disubstitution and found that this modification led to a loss of potency relative to the corresponding P1′ isobutyl compounds with the least detrimental effect being observed for a P1′ *gem*-cyclohexyl compound **35**.[164] However, a P1′ quaternary carbon is tolerated when one of the substituents is hydroxyl as in compound **36**[165] or when one of the substituents is cyclized onto the nitrogen of the P1′−P2′ amide as in compound **37**.[166]

Replacement of the P1′−P2′ amide bond of succinyl hydroxamic acid MMP inhibitors by a sulfonamide bond, as in compound **38**, results in a substantial loss of MMP inhibitory activity.[167] This has been explained in terms of the hydrogen bond from the N−H of the conserved leucine (Leu-160 for MMP-8) to the sulfonyl oxygen being less energetically accessible due to the pyramidal nature of the sulfonamide.[167] The P1′−P2′ amide has also been replaced by a urea functionality, but these analogues were found to be unstable and prone to acid-catalyzed hydantoin formation.[167]

c. P2′ Modifications. X-ray crystallographic analysis of MMP-inhibitor complexes reveals that the P2′ group of peptidyl succinyl hydroxamic acid based MMP inhibitors points out of the enzyme, making few contacts with the S2′ cleft.[76] Indeed, analysis of SAR indicates that modification of the P2′ group has, in general, a modest effect on in vitro activity. Tryptophan at P2′, as in GM6001 (**39**),[168,169] yields more potent inhibitors than other amino acid side chains.[170] The group at P2′ can, however, have an effect on the pharmacokinetic properties of the inhibitors. We have previously suggested that the oral activity of marimastat results from the beneficial combination of a sterically bulky *tert*-butyl group and an α-hydroxy group which increases aqueous solubility.[129] We argued that the bulky P2′ group shields the adjacent amide bonds reducing hydration[171] and, hence, the

desolvation energy barrier of the peptide backbone associated with absorption from an aqueous environment to the lipid environment of cell membranes.[172] A P2′ *tert*-butyl group is also a feature of the orally available compounds Ro 31−9790 (**40**)[173] and CT1746 (**41**).[174,175] The phamacokinetics of Ro 31−9790 has been studied in man, and it has been found that the main metabolite is the amide which arises from dehydroxylation of the hydroxamic acid moiety.[176] The deep pocket selective compound CT1746 has been shown to be effective in animal models of cancer following oral administration.[174,175] Babine and Bender suggest that the P2′ *tert*-butyl group is probably inferior with respect to VDW interactions compared to other, more extended side chains but that this is offset by more facile desolvation of the adjacent peptide linkages and a conformational effect that preorganizes the compound for binding.[76] Ikeda and co-workers describe compounds that feature a P2′ phenyl substituent, e.g., KB−R7785 (**42**).[177] KB−R7785 is orally active as determined both by an ex vivo MMP-1 inhibition assay in mice and demonstration of efficacy in a rat adjuvant arthritis assay. The beneficial effect of the P2′ phenyl group on absorption is attributed to it being an amide shielding moiety.[177] The same researchers have also investigated the SAR for the inhibition of an MMP-14 mutant lacking the transmembrane domain.[178] It was found that the phenylglycine derivative **42** was a weaker inhibitor of MMP-14 than the corresponding cyclohexylglycine derivative **43**, BB-94 (**1**), BB-2516 (**12**), or Ro 31−9790 (**40**). A homology model of MMP-14 suggests that the S1 and S2′ subsites are narrower than those of other MMPs.[178] This results in the phenylglycine compound **42** binding in a conformer which is not at an energy minimum whereas the cyclohexylglycine compound **43** and the *tert*-butylglycine compounds **12** and **14** can bind to MMP-14 in a low-energy comformation.[178]

The P2′ and P3′ substituents may be cyclized to form a lactam, and it is found that there is a correlation between the inhibitory potency and ring size for compounds with both hydroxamic acid and phosphonic acid ZBGs.[114,179] This has been attributed to trans amide geometry for the P2′−P3′ being the required geometry for effective hydrogen bonding interactions between enzyme and inhibitor. The inhibitory potency was found to increase as the lactam ring size was increased from 7 to 9 and then to 13 atoms.[114] Trans geometry is observed in the complex between MMP-8 catalytic domain and BB-1909 (**13**), which features a 13-membered lactam ring between P2′ and P3′.[135] Indolactam cyclization between P2′ and P3′ (e.g., **3**) also confers trans amide geometry and increases activity by at least 10-fold relative to the acyclic analogues.[124]

d. P3′ Modifications. The S3′ region of the MMP enzymes is a relatively open area and a wide range of groups may be introduced at P3′. Heteroaryl and aryl groups appear to enhance MMP-3 and TACE inhibitory activity. We found that the introduction of a benzhydryl group at P3′, e.g., **44**, leads to compounds that are selective for MMP-7 and MMP-3 relative to MMP-1 and MMP-2.[180,181] Disubstitution of the P2′−P3′ amide tends to reduce inhibitory activity,[114,179] except for P2′−P3′ caprolactam derivatives.[182] It has been found that the weak inhibitory activity of P2′−P3′ caprolactam derivatives unsubstituted at P3′[114] may be increased substantially by the introduction of a P3′ methyl acetate substituent as in compound **45**.[182] Interestingly, this compound is a selective inhibitor of MMP-1 over MMP-3 despite possessing an extended C_8 alkyl substituent at P1′. Removal of the P3′ ester carbonyl to provide the corresponding methyl ethyl ether results in a reversal of selectivity for inhibition of MMP-3 over that of MMP-1.[182] A P2′ dihydrocarbostyril derivative, OPB-3206 (**46**), exhibits weak MMP inhibitory activity but is orally available in the rat.[183]

The P2′−P3′ amide is not strictly required for inhibitory activity since it may be replaced by a variety of alternative functionalities. For example, the P2′ amino acid may by replaced by a β-amino alcohol, as in compound **32**, but this generally results in a 10−50-fold loss of activity.[76] From the X-ray crystallographic analysis of **32** complexed to the catalytic domain of MMP-3, it is apparent that the C-terminal hydroxy group accepts a hydrogen bond from the N−H of Tyr-240 (MMP-3 numbering).[76]

The replacement of the C-terminal amide group with a nitrogen heterocycle has been a successful modification. An X-ray crystal structure of a C-terminal imidazole **47** complexed to MMP-7 has been reported.[76,184] The use of imidazole to replace the C-terminal *N*-methyl amide is said to result in a 5-fold reduction in potency against all MMPs tested for this analogue of GM6001.[76] Examination of the crystal structure shows that the imidazole makes hydrogen bonds to the carbonyl of Asn-179 and to the N−H of Tyr-240 (MMP-7 numbering) without any apparent perturbation of the inhibitor backbone in comparison to a related structure in which the C-terminal *N*-methyl amide is retained.[76,184] The introduction of a phenyl substituent at the 5-position of the imidazole ring provided selective inhibition of MMP-7 over MMP-1 and MMP-3, whereas a P3′ benzimidazole group provided broad-spectrum inhibition of the three MMPs tested.[184]

The replacement of the P2′−P3′ amide by an aryl ketone or heteroaryl ketone group, as in the P3′ indole ketone **48** and the P1−P2′ cyclized P3′ phenyl ketone **49**, is tolerated.[185] Compound **48** is an analogue of BB-1101 (**11**) which possesses negligible oral availability. In contrast, the indole ketone **48** is 12% bioavailable in the monkey following dosing at 10 mg/kg p.o. and has a half-life of 20 h.[185] Two earlier studies of P2′−P3′ amide derivatives showed that the nature of the P3′ substituent can have an effect on the oral availability of succinyl hydroxamates.[186,187] In one study, the effect of P3′ substituents on biliary excretion in the rat was examined for a series of GM6001 analogues.[186] It was found that the presence of a tertiary amine at P3′ reduced biliary excretion and increased plasma half-life.[186] The tertiary amine **50** was found to be 8.5% orally bioavailable in the rat.[186] In the other study, it was found that oral availability as measured in a mouse pleural cavity assay was significantly enhanced by the introduction

of an alkyl morpholino P3′ substituent.[187] The beneficial effect of the morpholino group was attributed to its basicity.[187] Epimerisation-free amide coupling conditions have been developed by Fray and Ellis to facilitate the introduction of a wide range of P3′ substituents into a *N*-succinyl-*tert*-leucine intermediate for the preparation of succinyl hydroxamates.[188]

B. Non-Peptidic Succinyl Hydroxamates

Truncation of the P2′−P3′ group of pseudo-peptide succinyl hydroxamic acid derivatives leads to MMP inhibitors which tend to be selective for the collagenases. Broadhurst and co-workers discovered that potent inhibition of the collagenases could be achieved when a cyclic imide group is introduced at P1, as in the phthalimido derivative **51**.[189] Subsequent optimization of this series led to the discovery of Ro 32−3555 (**52**), which was selected for development for the treatment of arthritis.[190,191] Presumably, for compound **51** and Ro 32−3555 (**52**), a favorable balance between active-site interactions and solvation is maintained despite the removal of three hydrogen bonding groups in comparison to succinyl hydroxamates with P2′ groups. The presence of the cyclic imide group at P1, a feature of earlier MMP inhibitors identified by the same workers and by other groups (vide supra), appears to also be important for activity and may compensate for the loss of the hydrogen bonds as observed in the X-ray structure of Ro 32−0554 (**10**) complexed to the active site of MMP-1.[130] The cyclopentylmethyl P1′ group of Ro 32−3555 was chosen on the basis of X-ray crystal structure data for MMP-1.[190] The introduction of cyclopentylmethyl provides a modest increase in potency over an analogue of Ro 32−3555 with an isobutyl group at P1′ suggesting improved complementarity with the S1′ pocket.[190] Ro 32−3555 exhibits an oral bioavailability of 26% in the rat and inhibits articular cartilage degradation in a rat monoarthritis model.[192] Ro 32−3555 (Trocade) has been referred to as a cartilage protective agent (CPA).[193] An improved synthesis of the chiral 2,3-disubstituted succinate of Ro 32−3555 has been reported[194] based on the earlier succinate alkylation protocol of Crimmin and co-workers.[195] We found that incorporation of a sulfonamide moiety at P1 in combination with P2′−P3′ truncation, as in compound **53**, can provide selective inhibition of MMP-1 over other the MMPs that we evaluated.[196] Analogues of **53** possess different selectivity profiles depending on the nature of the sulfonyl substituent.

Alpegiani and co-workers found that an α amino group in conjunction with a P2′ piperazinyl moiety, as in compound **54**, provided compounds with good oral availability.[197] Compound **54** exhibits 58% oral bioavailability in the rat and 34% in the cynomolgus monkey.[197] Broadhurst and co-workers have found that the P2′−P3′ amino acid residue may be replaced by a hydrazide moiety as in Ro 32−7315 (**55**).[198,199] This potent TACE selective inhibitor has been selected for clinical development.[199] Cyclic hydrazide compounds such as the piperazic acid derivative matlystatin B (**56**) and its analogues have been previously identified as natural product MMP inhibitors.[155,200−202] Matlystatin analogues have been prepared in which the ZBG has been altered,[203] the P3′ group modified,[155,204] and the P1′ substituent changed.[155] As described above, the P1′ nonyl derivative **27** is a potent inhibitor of the gelatinases, in contrast to the modest activity exhibited by the parent molecule matlystatin B (**56**).[155]

The P2′ amino acid residue of succinyl hydroxamic acid MMP inhibitors may be replaced with a benzhydryl group, as in compound **57**.[111] A combinatorial chemistry approach was employed in this study that first involved the exploration of P2′ modifications for a series of *N*-carboxyalkyl amino acid based inhibitors (vide infra). Modeling of the optimal P2′ group that had been identified by this approach led to the identification of benzhydryl as a preferred substituent, which was then introduced into the corresponding succinyl hydroxamic acid derivative **57**. An X-ray crystal structure of this compound bound to the catalytic domain of MMP-3 revealed an unexpected conformational shift in the 222−231 loop region (MMP-3 numbering).[111] This illustrates that subtle changes in binding can occur with variation of inhibitor structure and that these are very difficult to predict on the basis of modeling alone. Inhibition of MMP-3 was further increased by replacing one of the phenyl groups of compound **57** with 3-pyridyl.[111]

C. Sulfonamide Hydroxamates and Related Structures

N-Sulfonyl amino acid hydroxamates were independently identified as inhibitors of MMPs by two research groups.[205,206] The first such compound to enter development is the orally available broad-spectrum inhibitor CGS 27023A (**58**).[205] Key structural features of CGS 27023A are said to be the isopropyl substituent which slows down metabolism of the adjacent hydroxamic acid group and the basic 3-pyridyl substituent which may aid partitioning into the hydrated negatively charged environment of cartilage.[205] SAR for the inhibition of macrophage metalloelastase (MMP-12) by CGS 27023A and analogues has been reported.[207] This reveals that CGS 27023A is a potent inhibitor of MMP-12, an enzyme that has been implicated in the development of emphysema that results from chronic inhalation of cigarette smoke.[208] The binding mode of CGS 27023A and analogues to MMP-3 has been investigated by NMR spectroscopy.[209−211] The *p*-methoxyphenyl substituent of CGS 27023A occupies, but does not fill, the S1′ specificity pocket, while the pyridylmethyl and isobutyl substituents occupy the S2′ and S1′ subsites, respectively.[211] An X-ray crystallographic analysis of a related compound CGS 25966 (**59**), a close analogue of CGS 27032A, complexed to the catalytic domain of MMP-1 has been reported by Babine and Bender.[76] The observed binding mode is broadly in agreement with the NMR studies in that the 4-methoxyphenyl group resides in the S1′ pocket and the isopropyl group is located in the S1 subsite. The X-ray structure indicates that the isopropyl group is relatively close to the *N*-benzyl substituent.[76] By introducing a six-membered ring to provide beneficial ligand preorganization[76] and extending the P1′ sub-

stituent, the potent MMP inhibitor AG3340 (**60**) was derived.[212,213] This compound was selected for development based on its superior efficacy in a murine model of cancer growth and metastasis in comparison to a number of analogues and because it showed a favorable pharmacokinetic profile with 18% oral bioavailability in rats.[213] There has been considerable interest from other researchers in analogues of CGS 27023A and AG3340.[141] For example, it has been recently reported by Hanessian and co-workers that modification of the substituent α to the hydroxamic acid in CGS 27023A leads to increases in the inhibition of the deep pocket MMPs, e.g., thioether derivative **61**.[214] Other analogues of particular interest are sulfone derivatives,[215-218] bis-sulfonamides,[219,220] and phosphinamides.[221] Groneberg, Burns, and co-workers have identified sulfone hydroxamic acids (e.g., **62**) which are inhibitors of both MMPs and the enzyme phosphodiesterase type 4 (PDE4).[216] Inhibition of PDE4 results in increased intracellular concentration of cyclic AMP and consequently in antiinflammatory activity.[222] Analogues of compound **62** have been identified, by both combinatorial methods (vide infra)[215] and traditional analogue synthesis,[216] that provide selective inhibition of PDE4 over MMP inhibition by the introduction of a 3,4-dimethoxyphenylsulfonyl group. The reverse selectivity for MMP inhibition over that of PDE4 is achieved by the incorporation of a cyclic quaternary center α to the sulfonyl moiety.[215] This is a structural feature of the compound RS-113,456 (**63**) identified by Campbell and co-workers.[217,218] Oral availability and half-life were improved in this series by shifting the cyclic group to be α to the hydroxamic acid as in the development compound RS-130,830 (**64**).[217] Separate X-ray crystallographic analyses of both RS-113,456 and RS-130,830 bound to the catalytic domain of MMP-13 reveal that the two compounds adopt virtually identical conformations.[91] An X-ray crystal structure has also been determined for RS-104,966, an analogue of **63** lacking the chloro substituent, bound to the catalytic domain of MMP-1. This shows that induced fit of MMP inhibitors with large P1′ substituents can occur by Arg-214 (MMP-1 numbering) adopting a new position, creating a larger open S1′ pocket.[91] RS-113,456 (**63**) dosed orally diminishes flow-mediated arterial enlargement in a rat arteriovenous fistula model,[217] and RS-130,830 (**64**) is being investigated in the clinic as a therapeutic agent for the treatment of osteoarthritis.[218] In contrast to the majority of MMP inhibitors, both RS-113,456 and RS-130,830 lack any stereocenters yet retain potent inhibitory activity for the deep pocket MMPs. In an alternative approach based on symmetrical bis-sulfonamides, Pikul and co-workers identified another series of nonchiral MMP inhibitors (e.g., **65**).[219] The 1,3-piperazinyl derivative PGE−4410186 (**65**) exhibits broad-spectrum inhibitory activity against the enzymes tested.[219] An X-ray crystal structure of PGE−4410186 complexed to the catalytic domain of MMP-3 reveals similar interactions previously observed in the crystal structure of CGS 25966 complexed to the catalytic domain of MMP-1[76] in terms of one of the 4-methoxyphenylsulfonyl groups residing in the S1′ pocket.[219] The second 4-methoxyphenylsufonyl binds to the S1/S2 pocket with the two sulfonyl oxygen atoms of this group interacting with the imidazole ring of His-166 (MMP-3 numbering) via a hydrogen-bonded bridging water molecule.[219] PGE−4410186 and analogues have been evaluated in an in vitro cartilage permeation model.[220] It was found that permeability across articular cartilage was increased for analogues of PGE−4410186 with increasing hydrophilicity.[220] Pikul and co-workers have also investigated analogues of CGS 27023A in which the sulfonamide moiety is replaced by a phosphinamide group as in compound **66**.[221] This compound is a potent inhibitor of MMP-3, the collagenases (MMP-1, MMP-8, MMP-13), and the gelatinases (MMP-2, MMP-9) but is less effective at inhibiting MMP-7. An X-ray crystal structure of phosphinamide **66** bound to MMP-3 catalytic domain reveals that the phosphinamide phenyl group is accommodated into the S1′ pocket and that the phosphinamide oxygen is within hydrogen bonding distance to the N−H of Leu-164 and Ala-165 (MMP-3 numbering).[221] This provides an explanation for the observation that optimum enzyme inhibitory activity is achieved when the configuration at the phosporus chiral center is *R*.[221] However, hydrolysis of the phosphinamide bond which occurs at low pH may limit the potential of these compounds to be developed into orally available drugs.

A new drug discovery technique involving multidimensional NMR spectroscopy, known as "SAR by NMR", was first used to identify potent analogues of the immunosuppressant FK506.[223] Application of this technique has been extended to the field of MMP inhibition, where it has been used to identify a series of MMP-3 inhibitors.[224,225] In this study, two ligands that bind weakly to proximal sites on MMP-3 were identified. Acetohydroxamic acid binds to the active-site zinc(II) ion and 3-cyanomethyl-4′-hydroxybiphenyl binds to the S1′ pocket.[224] On the basis of the NMR-derived structural information, the two molecules were linked together to give the potent MMP-3 inhibitor **67**.[224]

D. Non-Hydroxamates

Due to the intense competition in the area of hydroxamic acid MMP inhibitors there has been considerable interest in compounds with alternative zinc binding groups. For the purpose of this review these are subdivided as follows: (1) Carboxylic acid and N-carboxyalkyl ZBGs, (2) Thiol ZBGs, (3) Phosphorus-based ZBGs, (4) Novel zinc binding groups.

1. Carboxylic Acids and N-Carboxyalkyl ZBGs

With the exception of a few families of hydroxamic acids derived directly by solid-phase procedures, all the other examples of MMP inhibitors described in the preceding sections will have been prepared by converting a carboxylic precursor into the corresponding hydroxamic acid. Consequently, a vast volume of test data has been built up on the value of carboxylic acid containing structures as potential MMP inhibitors. A typical example is the carboxylic acid precursor of compound **29**; this is selective for MMP-2 over MMP-1, -3, and -7 (IC_{50} of 30 nM vs

APPENDIX 3 別紙1

R1,R2,R6,R7,R8 : hydrogen

Compounds	Inhibitory Activity (IC50, nM)			R3	R4	R5	R9	X	Y	
	MMP-1	MMP-3	TNF-α Convertase						A	B
25	10	80	4023	methyl	isobutyl	methyl	guanidoethyl	ethylene	imino	amidino
28	10	120	2500				guanidomethyl	methylene		
38	62	180					aminopropyl	trimethylene	—	amino
41	22	18	1012				4-acetimidoylimino-methylphenyl	phenylene	methylene-imino	acetimidoyl
22	52	120	2080				acetimidoyliminopropyl	trimethylene	imino	acetimidoyl
45	56	100 *	2400 *				propionimidoyliminopropyl			propionimidoyl
77	32	56 *	740 *				benzimidoyliminopropyl			benzimidoyl
29	17	110					aminoethyl	ethylene	—	amino
23	17	16	1193				4-aminomethylphenyl	phenylene	methylene	amino
33	17	12	330				4-tetraethyl bis(phosphono) methyliminophenyl	phenylene	imino	4-tetraethyl bis(phosphono) methyl
31	12	11	3100				4-tetramethyl bis(phosphono) methyliminophenyl			4-tetramethyl bis(phosphono) methyl
34	13	11	1040				4-triethyl bis(phosphono) methyliminophenyl			4-triethyl bis(phosphono) methyl
32	17	12	1500				4-trimethyl bis(phosphono) methyliminophenyl			4-trimethyl bis(phosphono) methyl
39		220 *		methyl	isobutyl	2-hydroxy-1-methylethyl	acetimidoyliminopropyl	trimethylene	imino	acetimidoyl
40		180 *				piperidyl				
37	42	50	7800	methyl	isobutyl	cyclopropyl	aminoethyl	ethylene	—	amino
42	230	24	8600			morpholinyl	4-acetimidoylimino-methylphenyl	phenylene	methylene-imino	acetimidoyl
85	12	18	2000	isobutyl	isobutyl	2-carboxyethyl	4-acetimidoylimino-methylphenyl	phenylene	methylene-imino	acetimidoyl
84	11	20	2200				4-aminomethylphenyl		methylene	amino
90	42	30	1800			2-(N',N'-dimethylamino) ethyl	4-acetimidoylimino-methylphenyl		methylene-imino	acetimidoyl
89	50	25	1900				4-aminomethylphenyl		methylene	amino
91	17	19	1800			2-hydroxyethyl	4-acetimidoylimino-methylphenyl		methylene-imino	acetimidoyl
35	22	19	1800				4-aminomethylphenyl		methylene	amino
93	48	36 *				2-hydroxy1,1-dimethylethyl	4-acetimidoylimino-methylphenyl	phenylene	methylene-imino	acetimidoyl
24	5	18	480			methyl	guanidoethyl	ethylene	imino	amidino
27	7	60	1000				guanidomethyl	methylene		

Compounds	Inhibitory Activity (IC50, nM)			R3	R4	R5	R9	X	Y	
	MMP-1	MMP-3	TNF-α Convertase						A	B
74	131	62	603	phenylpropyl	isopropyl	methyl	aminopropyl	trimethylene	—	amino
56		595	697 *		2-naphthyl-methyl		guanidoethyl	ethylene	imino	amidino
26	20	40	960		isobutyl	methyl	guanidomethyl	methylene	imino	amidino
47	5	8	210				4-aminomethylphenyl	phenylene	methylene	amino
44	32	17	129		isobutyl	2-(N',N'-dimethylamino) ethyl	acetimidoyliminopropyl	trimethylene	imino	acetimidoyl
36	14	9	190			2-hydroxyethyl				
30	10	12	190			methyl	~~acetimidoyliminopropyl~~			
73	11	52	3028	morpholinopropyl	isobutyl	methyl	aminopropyl	trimethylene	—	amino
67	5	14	527	carboxyphenylpropyl						
75	14	10	494							
61	15	23	1098	aminomethylphenylpropyl						
72	5	11	250	hydroxyphenylpropyl						
65	9	18	421	methoxycarbonylphenylpropyl						
71	4	10	352							
76	33	113 *	5480	piperidinopropyl						
70	89	28	854	iso-butyloxyethyl						
69	36	20	1085	butoxyethyl						
66	23	59 *	3254	ethoxyethoxyethyl						
68	47	14	534	hydroxyoctyl						
83		14 *	2735	isobutyl	8-hydroxyoctyl	methyl	aminopropyl	trimethylene	—	amino
82	4	6	637	(3,4,4-trimethyl-2,5-dioxo-imidazolidin-1-yl)propyl	isobutyl	methyl	aminopropyl	trimethylene	—	amino
58	41	51	942	aminomethylphenylpropyl	isobutyl	methyl	hydrogen	hydrogen	—	—
60	31	66	1214							
53	253	647	8430	aminomethylbenzyl						
57	235	808								
62	138	179	2893	acetimidoyliminopentyl						
63	137	214 *	4579	isopropyliminopentyl						
64	91	86 *	3670	(S)5-(pyridin-4-ylmethyl-imino)pentyl						
59	217	840	9949	isopropyliminomethylbenzyl						
54	14	10	340	phenoxyethyl	isobutyl	methyl	guanidoethyl	ethylene	imino	amidino
55	18	9	279	cyclohexylpropyl						
50	13	9	599	(p-toluenesulfonamido-methyl)benzyl						
52	15	23	1660	p-methanesulfonamido-methylbenzyl						
51	9	2	426	p-phthalimidomethylbenzyl						

ompounds	Inhibitory Activity (IC50, nM)			R3	R4	R5	R9	X	Y	
	MMP-1	MMP-3	TNF-α Convertase						A	B
80	9	60	1248	aminomethylphenylpropyl	isobutyl	methyl	aminoethyl	ethylene	—	amino
78	18	21	705				aminomethyl	methylene	—	amino
106		691	3707		isopropyl		aminoethyl	ethylene	—	amino
92	64	52		aminophenylpropyl	isobutyl	2-(N',N'-dimethylamino) ethyl	4-acetimidoyliminopropyl	trimethylene	imino	acetimidoyl
46	15	42	460	guanidophenylpropyl	isobutyl	methyl	aminoethyl	ethylene	—	amino
81	13	21	737	guanidomethylphenylpropyl						
48	32	250	7600	(S)4-aminomethylbenzyl			aminoethyl			
49	25	68 *	5000	(S)4-aminomethylbenzyl	isobutyl	methyl	4-aminomethylphenyl	phenylene	methylene	amino

□:今回出すデータ : Disclosed concurrently with this Response

▨:明細書に記載あり : Disclosed in the Specification

□*:1st Actionに対する応答書に記載 Mentioned in our Response to the 1st Official Action